Efficient and facile synthesis of pyrazoles using Guar-gum as biocatalyst and their *in vitro* bio-evaluation

Suman Sangwan (✉ sangwansuman99@gmail.com)

Chaudhary Charan Singh Haryana Agricultural University

Rajvir Singh

CCS HAU: Chaudhary Charan Singh Haryana Agricultural University

SUSHEEL GULATI

CCS Haryana Agricultural University: Chaudhary Charan Singh Haryana Agricultural University

<https://orcid.org/0000-0002-2989-6758>

Suprita Rana

CCS HAU: Chaudhary Charan Singh Haryana Agricultural University

Research Article

Keywords: Pyrazoles, Guar-gum, Raphanus sativus L., Rhizoctonia solani, Aspergillus niger

Posted Date: May 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-488723/v1>

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Abstract

A green efficient and facile synthesis of pyrazoles is reported *via* condensation reaction between substituted aldehydes (**1a-1i**), malononitrile (**2**) and 2,4-dinitrophenyl hydrazine (**3**) in presence of acylated Guar-gum as biocatalyst under solvent-free conditions. The progress of reaction was checked by thin layer chromatography and melting points reported are uncorrected. All synthesized compounds (**4a-4i**) were characterized by using ^1H NMR and FTIR spectral techniques and evaluated for *in vitro* herbicidal activity against *Raphanus sativus* L. (Radish seeds). All compounds (**4a-4i**) were also evaluated for their antifungal activity against *Rhizoctonia solani* and *Aspergillus niger* by poisoned food techniques method. Antioxidant activity of synthesized compound was also determined. From activity results, it was found that compound **4f** was most active against both *Raphanus sativus* L. (root) and *Raphanus sativus* L. (shoot) respectively. Compounds **4e** and **4h** were found most active against *Rhizoctonia solani* and *Aspergillus niger* fungus respectively at highest concentration. Compound **4e** has shown maximum percentage DPPH free radical scavenging activity i.e. 61.47% at 100 $\mu\text{g/mL}$ concentration. Less reaction time, excellent yield of products, mild reaction conditions and simple work-up are some merits of present methodology.

Introduction

Recently organic chemists are attracted toward the development of eco-friendly approach for synthesis of biological active heterocyclic functionalities. The development of simple synthetic methods for widely used organic compounds from readily available starting materials is one of the major challenges for organic chemist¹. Nowadays the use of the biocatalyst for organic synthesis is highly safe that had gained a precious scope in literature. This area of chemistry is a new thought which involves isolation, characterization and synthesis of new compounds and focus mainly on environment safety. Among all the green chemistry aspects selection of green catalyst is an important part of chemical reaction². Nitrogen containing heterocyclic compounds is widely occurring in nature and their applications to biologically active pharmaceuticals, agrochemicals and functional materials are gaining more importance. Thus the development of waste minimized synthetic methods for nitrogen containing heterocycles with structural diversity is one of major field of interest for organic chemists. Among N-heterocycles pyrazoles is important biologically active heterocyclic compounds and part of natural products. They are mainly used in supramolecular and polymer chemistry, pharmaceuticals, agrochemical industry, food, cosmetic colouring, complexing agents for the synthesis of hydrogenation catalysts and UV stabilizers³⁻⁸. Various methods have been developed for the synthesis of pyrazoles, but most of these methods are not compatible with the environment and also expensive. Therefore, the development of simple and efficient synthesis of pyrazole ring under eco-friendly conditions is highly appreciated⁹⁻¹⁰. In this paper, we reported the synthesis of pyrazoles *via* one-pot three component reaction between substituted aldehydes, malononitrile and 2,4-dinitrophenylhydrazine in the presence of Guar-gum. The development of new biocatalysts for synthesis of heterocyclic compounds helps to maximize prevention of waste, high atom economy of products and reduce the use of hazardous chemicals.

Experimental

All chemicals and solvent used were of analytical grade. Melting points were determined on Ganson electric melting point apparatus and are uncorrected. The homogeneity of the reaction was confirmed by (TLC) thin layer chromatography. The ^1H NMR spectra were recorded in CDCl_3 or $\text{DMSO}-d_6$ using tetramethyl silane (TMS) as internal reference on "Brucker Ac 400 F"(400MHz) nuclear magnetic resonance spectrometer. The chemical shifts values were quoted in parts per million. The following abbreviations correlate with the multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, m = multiplet and brs = broad singlet. Infrared spectra ($4000\text{-}350\text{ cm}^{-1}$) of the synthesized compounds were recorded in KBr pellets on Perkin Elmer FT-IR-R2X spectrophotometer and frequency was expressed in cm^{-1} .

Acylation of Guar-Gum

Take 2 g Guar-gum in 100 mL distilled water in a 250 mL flask and stirred on magnetic stirrer. After the gum was well dispersed, then 10 mL of NaOH solution was added at a rate of 1 mL within 15 min with constant stirring at room temperature. An aliquot of 10 mL chloro-acetic acid was then added to the reaction mixture over a period of 10 min. The reaction mixture was heated at $65\text{ }^\circ\text{C}$ with constant stirring for 4 hours to drive the reaction process to completion. The product was separated with ethanol and then added several drops of glacial acetic acid. Finally the precipitate was washed with water and finally freezes and dried¹¹.

Chemistry of Acylated Guar-Gum

Bioevaluation

Screening of herbicidal activity

Solutions of 50 µg/ mL, 100 µg/ mL, 150 µg/ mL and 200 µg/ mL of the test compounds in DMSO were prepared. Agar powder (5g) was put into boiling distilled water (1L) until it dissolved, and then cooled down to 40-50°C. The solution (2 mL) containing test compounds and melting agar (18 mL) was mixed and this mixture was added to a Petridish with 4.5 cm diameter. The agar plate without test compound was used as an untreated control. Then 15 seeds of *Raphanus Sativus* L. (Radish) were put on the surface of the agar plate. The Petridishes were covered with glass lids, and the cultivation conditions were kept at 25±1 °C and 12 hours in light and 12 hours in dark alternating for seven days. Seven days later, the root lengths and shoot lengths of *Raphanus sativus* L. were measured. The growth inhibitory rate related to untreated control was determined by given formula¹².

$$\% \text{ inhibition} = ((\text{Control} - \text{Treated}) / \text{Control}) \times 100$$

Screening of antifungal activity

Amongst the various methods available, poisoned food technique¹³ which is the most common was used for testing antifungal activity. The test fungus was grown on Potato dextrose agar medium. The required amount of synthesized compounds dissolved in 1 mL of DMSO was incorporated aseptically into 99 mL aliquots of sterilized potato dextrose agar cooled at 45° C after brief shaking. Each lot of medium was poured into Petri dishes and allowed to solidify. 1 mL DMSO in media was taken as control. Each dish was inoculated centrally with a 5 mm mycelial disc cut from the periphery of 2-3 days old fungal colonies. Inoculated Petri plates were incubated in the dark 25±2°C for 48-72 h and colony diameters were measured periodically till the control dishes were nearly completely covered with fungus growth. Three replicates were used for each concentration of a chemical together with three dishes containing only the solvent and no toxicant. The degree of inhibition of growth was calculated from the mean differences between treatments and the control as percentage of latter by using the formula.

$$\% \text{ inhibition} = ((\text{Control} - \text{Treated}) / \text{Control}) \times 100$$

Control = mycelial growth in control dish

Treated = mycelial growth in treated dish

Screening of antioxidant activity

Solutions of 25 µg/ mL, 50 µg/ mL, 75 µg/ mL and 100 µg/ mL, of the tested compounds in methanol were prepared. For evaluation of antioxidant activity in 0.2 mL of concentration solution, 3.0 mL of 2,2-diphenyl-1-picrylhydrazyl solution was added and mixed thoroughly for 5 min. A control was also made containing 0.2 mL of solvent without concentration of compounds. The absorbance of sample as well as control was measured at 517 nm after 30 min of incubation in dark at room temperature using the UV-visible double

beam spectrophotometer model 2203 (Systronics Cop.) against a blank containing respective solvent. A graph was drawn by plotting percent DPPH free radical scavenging activity (y-axis) against given compound concentration (x-axis). Then using the Microsoft Excel Software, a quadratic regression equation ($y = ax^2 + bx + c$) was obtained. By putting $y = 50$ in the equation $y = ax^2 + bx + c$, it was converted to the form $ax^2 + bx + c = 0$, IC_{50} was calculated from the equation above given and using the formula given below:

$$X = -b \pm \sqrt{b^2 - 4ac} / 2a$$

Where $X = IC_{50}$ ($\mu\text{g/mL}$)

Calculation of the percentage of DPPH scavenged was calculated by using the formula

$$\% \text{ DPPH free radical scavenging activity} = ((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100$$

Where,

A_{control} = Absorbance of control

A_{sample} = Absorbance of sample

General method for the synthesis of substituted pyrazoles (4a-4i)

A mixture of substituted aldehydes (**1a-1i**) (20 mmole), malononitrile (**2**) (20 mmole) and 2,4-dinitrophenyl hydrazine (**3**) (20 mmole) was stirred at room temperature in presence of Guar-gum. The progress of the reaction was monitored with the help of thin layer chromatography. After completion of the reaction, the separated solid was filtered and washed with cold water to get the product (**4a-4i**), which was further recrystallized with ethylacetate and then characterized by ^1H NMR and FTIR spectroscopy.

Results And Discussion

The synthesis of pyrazole derivatives (**4a-4i**) were carried out by reaction between 4-methoxy benzaldehyde (**1a**), 4-chloro benzaldehyde (**1b**), 4-methyl benzaldehyde (**1c**), 2-chloro benzaldehyde (**1d**), 3-nitro benzaldehyde (**1e**), 2-hydroxy benzaldehyde (**1f**), 4-hydroxy benzaldehyde (**1g**), 3,4-hydroxy benzaldehyde (**1h**), 3-hydroxy benzaldehyde (**1i**) with malononitrile (**2**) and 2,4-dinitrophenylhydrazine (**3**) in presence of Guar-gum as biocatalyst (**Scheme 1**). The progress of reaction was monitored by thin layer chromatography using Hexane: Ethyl acetate (80:20, v/v) as an eluent.

After completion of the reaction, the solid products were collected by simple filtration and then recrystallized with ethylacetate to afford pure pyrazoles derivatives (**4a-4i**) as shown in Fig. 1. The physical data of substituted pyrazoles derivatives (**4a-4i**) was shown in Table 1. The structure of synthesized compounds was confirmed by ^1H NMR, FTIR spectral analysis as well as comparison of their melting points with those of reported compounds. The optimization of reaction conditions has been presented in

Table 2. The comparison of activity of different catalysts with respect to time and yield of reaction as shown in Table 3. It was found that Guar-gum gives the best catalytic activity in terms of products yield, reaction conditions and reaction time as compared to other catalyst in literature *viz.* I_2/K_2CO_3 , $ZnCl_2$, Al_2O_3 , NaH , Chloranil and Glycine. The catalyst used in present study is nature derived, easily available and inexpensive which makes this procedure eco-friendly. The plausible mechanism for the formation of substituted pyrazoles derivatives (**4a-4i**) is shown in Scheme 2. First, nitrile ion is activated by removal of acidic hydrogen from malononitrile catalyzed by acidic catalyst. Finally, the arylidene nitrile intermediate formed through the Knoevenagel condensation reaction between the intermediate nitrile anion and substituted aldehydes. On the other hand, the reaction of 2,4-dinitrophenyl hydrazine with arylidene nitrile afforded an intramolecular cyclized product.

Table 1
Physical data of substituted pyrazole derivatives (**4a-4i**)

S. No	Products	Ar	Guar-gum		m.p. (°C)
			Time (h)	Yield (%)	
1	4a	4-OCH ₃	0.5	89	228–229, (Lit. 229–230) ¹⁴
2	4b	4-Cl	0.3	86	177–179
3	4c	4-CH ₃	0.5	82	184–185
4	4d	2-Cl	0.4	80	167–168, (Lit. 168) ¹⁵
5	4e	3-NO ₂	1.0	86	179–180
6	4f	2-OH	0.3	81	233–235
7	4g	4-OH	0.5	79	187–188
8	4h	3,4-OH	0.5	81	215–216
9	4i	3-OH	1.0	80	243–245

Table 2
Optimization of reaction conditions

Entry	Amount of Catalyst (g)	Time (h)	Yield (%)
1	0.5	3.0	70
2	1.0	3.0	72
3	2.0	2.5	72
4	3.0	2.2	82
5	4.0	2.1	78
6	5.0	2.1	78
7	6.0	2.0	76

Table 3
Comparison of the results of the present methods used for synthesis of pyrazoles with the reported methods in the literature

S. No.	Catalyst	Solvent	Temperature (°C)	Time (h)	Yield (%)	References
1	I ₂ /K ₂ CO ₃	THF	100	40	65	16
2	ZnCl ₂	THF	80	14	65	17
3	Al ₂ O ₃	DMF	RT	10	72	18
4	NaH	THF	100	20	66	19
5	Chloranil	Toluene	RT	4	71	20
6	Glycine	DMSO	RT	9	80	21
7	Guar-gum	Solvent-free	RT	4	82	Current work

Table 4
Herbicidal activity of substituted pyrazoles (**4a-4i**)

Products	Growth inhibition (%)							
	Root				Shoot			
	50 (µg/mL)	100 (µg/mL)	150 (µg/mL)	200 (µg/mL)	50 (µg/mL)	100 (µg/mL)	150 (µg/mL)	200 (µg/mL)
4a	31.00	47.03	51.97	87.94	47.11	52.06	63.02	77.00
4b	35.63	49.83	57.81	88.73	43.03	57.42	67.31	85.00
4c	31.42	52.76	56.00	82.00	18.01	36.13	67.01	78.07
4d	34.69	46.00	61.77	89.00	41.05	53.91	77.26	83.01
4e	27.81	37.00	72.41	86.04	43.01	61.00	74.72	84.00
4f	21.04	32.39	63.42	91.00	43.81	58.00	67.01	89.07
4g	36.97	43.28	51.89	69.72	32.24	54.17	66.16	82.00
4h	41.03	51.18	63.02	76.83	22.36	39.18	68.03	76.06
4i	30.75	64.00	75.00	89.03	32.15	47.00	78.01	83.11

Table 5
Antifungal activity of substituted pyrazoles (**4a-4i**)

Products	Growth inhibition (%)							
	Fungi							
	Rhizoctonia solani				Aspergillus niger			
	250 (µg/mL)	500 (µg/mL)	1000 (µg/mL)	2000 (µg/mL)	250 (µg/mL)	500 (µg/mL)	1000 (µg/mL)	2000 (µg/mL)
4a	37.78	47.12	58.68	69.55	40.13	49.18	58.24	66.68
4b	40.65	52.02	61.72	70.03	39.81	48.34	58.13	69.12
4c	38.75	49.52	60.00	70.72	41.23	51.00	60.78	69.85
4d	a	a	A	a	37.83	48.12	58	68.75
4e	41.52	51.22	62.00	70.88	40.85	49.52	57.26	68.81
4f	39.89	49.53	58.64	68.01	38.71	48.00	56.71	67.00
4g	40.72	49.53	58.65	69.71	a	A	a	a
4h	35.17	47.00	56.12	66.89	42.12	52.11	62.00	71.09
4i	39.67	48.14	58.00	69.12	40.05	49.54	58.13	69.00

Table 6: Antioxidant activity of substituted pyrazoles (**4a-4i**)

Products	DPPH free radical scavenging activity (%)				IC ₅₀ (µg/mL)
	25 (µg/mL)	50 (µg/mL)	75 (µg/mL)	100 (µg/mL)	
4a	7.27	11.01	23.11	32.12	122.30
4b	a	a	a	a	a
4c	a	a	a	a	a
4d	12.21	32.67	47.27	52.67	92.34
4e	16.36	24.72	48.10	61.47	87.14
4f	7.51	19.57	31.45	58.41	96.36
4g	a	a	a	a	a
4h	6.21	19.41	21.42	34.36	150.94
4i	a	a	a	a	a

a: no growth inhibition

Characterization data of synthesized compounds

5-amino-1-(2,4-dinitrophenyl)-3-(4-methoxyphenyl)-1H-pyrazole-4-carbonitrile (4a): m.p. 228-229°C [Lit. 229-230°C, Weber (2002)]; ¹H NMR (400 MHz, CDCl₃): δ 2.54 (s, 3H, OCH₃); 6.09-7.09 (m, 3H, Ar-H); 7.11-7.59 (m, 4H, Ar-H); 9.67 (s, 2H, NH₂); IR (ν_{max} cm⁻¹) (neat): 3237.0 (NH), 3031.0 (C=CH, aromatic), 2296.0 (CN), 1616.0 (C=C, aromatic), 1335.0 (NO₂)

5-amino-3-(4-chlorophenyl)-1-(2,4-dinitrophenyl)-1H-pyrazole-4-carbonitrile (4b): m.p. 177-179°C; ¹H NMR (400 MHz, CDCl₃): δ 7.39-7.67 (m, 3H, Ar-H); 7.21-7.57 (m, 4H, Ar-H); 8.72 (s, 2H, NH₂); IR (ν_{max} cm⁻¹) (neat): 3259.0 (NH), 3107.0 (C=CH, aromatic), 2223.0 (CN), 1616.0 (C=C, aromatic), 1335.0 (NO₂), 744.0 (C-Cl)

5-amino-1-(2,4-dinitrophenyl)-3-(4-methylphenyl)-1H-pyrazole-4-carbonitrile (4c): m.p. 183-184°C; ¹H NMR (400 MHz, CDCl₃): δ 2.42 (s, 3H, CH₃); 7.32-7.44 (m, 3H, Ar-H); 7.66-7.83 (m, 4H, Ar-H); 8.13 (s, 2H, NH₂); IR (ν_{max} cm⁻¹) (neat): 3262.0 (NH), 3110.0 (C=CH, aromatic), 2221.0 (CN), 1614.0 (C=C, aromatic), 1331.0 (NO₂)

5-amino-3-(2-chlorophenyl)-1-(2,4-dinitrophenyl)-1H-pyrazole-4-carbonitrile (4d): m.p. 167-168°C [Lit. 168°C, Hawang *et al.*, (2011)]; IR (ν_{max} cm⁻¹) (neat): 3288.0 (NH), 3052.0 (C=CH, aromatic), 2229.0 (CN), 1614.0 (C=C, aromatic), 1336.0 (NO₂), 758 (C-Cl)

5-amino-1-(2,4-dinitrophenyl)-3-(3-nitrophenyl)-1H-pyrazole-4-carbonitrile (4e): m.p. 179-180°C; IR (ν_{max} cm⁻¹) (neat): 3267.0 (NH), 3110.0 (C=CH, aromatic), 2228.0 (CN), 1614.0 (C=C, aromatic), 1335.0 (NO₂)

5-amino-1-(2,4-dinitrophenyl)-3-(2-hydroxyphenyl)-1H-pyrazole-4-carbonitrile (4f): m.p. 233-235°C; ¹H NMR (400 MHz, CDCl₃): δ 7.41-7.55 (m, 3H, Ar-H); 7.69-7.80 (m, 4H, Ar-H); 8.15 (s, 2H, NH₂); IR (ν_{max} cm⁻¹) (neat): 3483.0 (OH), 3293.0 (NH), 3112.0 (C=CH, aromatic), 2241.0 (CN), 1614.0 (C=C, aromatic), 1332.0 (NO₂)

5-amino-1-(2,4-dinitrophenyl)-3-(4-hydroxyphenyl)-1H-pyrazole-4-carbonitrile (4g): m.p. 187-188°C; IR (ν_{max} cm⁻¹) (neat): 3498.0 (OH), 3310.0 (NH), 3108.0 (C=CH, aromatic), 2221.0 (CN), 1616.0 (C=C, aromatic), 1316.0 (NO₂)

5-amino-1-(3,4-dihydroxyphenyl)-1-(2,4-dinitrophenyl)-1H-pyrazole-4-carbonitrile (4h): m.p. 214-215°C; ¹H NMR (400 MHz, CDCl₃): δ 7.14-7.19 (m, 3H, Ar-H); 7.13-8.03 (m, 3H, Ar-H); 8.12 (s, 2H, NH₂); IR (ν_{max} cm⁻¹) (neat): 3816.0 (OH), 3292.0 (NH), 2941.0 (C=CH, aromatic), 2226.0 (CN), 1623.0 (C=C, aromatic), 1332.0 (NO₂)

5-amino-1-(2,4-dinitrophenyl)-3-(3-hydroxyphenyl)-1H-pyrazole-4-carbonitrile (4i): m.p. 243-245°C; ¹H NMR (400 MHz, CDCl₃): δ 7.10-7.18 (m, 3H, Ar-H); 7.12-8.07 (m, 4H, Ar-H); 8.16 (s, 2H, NH₂); IR (ν_{max} cm⁻¹) (neat): 3481.0 (OH), 3292.0 (NH), 3112.0 (C=CH, aromatic), 2241.0 (CN), 1614.0 (C=C, aromatic), 1332.0 (NO₂)

Herbicidal activity

All synthesized compounds (**4a-4i**) were tested for herbicidal activity against *Raphanus sativus* L. at 200, 150, 100 and 50 µg/mL concentrations (Table 4). Results were shown in the form of primary screening. All compounds were diluted to 1000 µg/mL concentration as a stock solution. Herbicidal activity of compounds was evaluated against *Raphanus sativus* L. by inhibitory effect of compounds on the growth of weed roots and shoots. The percentage of inhibition growth was calculated from mean differences between treated and control. From the herbicidal activity data, we observed that compound **4f** was exhibited maximum percentage growth inhibition i.e. 91.00 against *Raphanus sativus* L. (root) and also compound **4f** was exhibited maximum percentage growth inhibition i.e. 89.07 against *Raphanus sativus* L. (shoot) respectively at 200 µg/mL concentration. This growth inhibition may be attributed to substitution of hydroxy group on phenyl ring. The box plot and graphical representation of herbicidal activity of all synthesized compounds (**4a-4i**) against *Raphanus sativus* L. were shown in Figs 2-5.

Antifungal activity

All synthesized compounds (**4a-4i**) were screened for their *in vitro* antifungal activity against *Rhizoctonia solani* and *Aspergillus niger*. The percentage growth inhibition of compounds against *R. Solani* and *A. niger* is presented in Table 5. DMSO was used as control against both the test fungi. A culture of test fungi was grown on Potato Dextrose Agar (PDA) medium at ambient temperature (25 ± 2°C). The stock solution (2000 µg/mL) of test compounds were prepared in DMSO and further dilutions were made to 1000, 500

and 250 µg/mL concentrations and stored at 4°C for further use. Potato Dextrose Agar Media, containing specific concentration of test compounds was poured on Petri plates. After solidification, small disc (0.5 cm diameter) of the fungus culture was cut with a sterile cork borer and transferred aseptically upside down in centre of Petri plate. Petri plates were incubated in BOD incubator at $25 \pm 2^\circ\text{C}$. Growth of fungal colony was measured after every 24 h till the fungus in control plates (containing DMSO) completely occupied it. From the fungicidal activity results, we concluded that compounds **4e** and **4h** were found to be most likely against *R. solani* and *A. niger* respectively. This result may be due to presence of nitro and hydroxy groups on phenyl ring. The box plot and graphical representation of antifungal activity of all synthesized compounds (**4a-4i**) against *Rhizoctonia solani* and *Aspergillus niger* were shown in Figs 6-9.

Antioxidant activity

The antioxidant activity data (Table 6) revealed that compounds **4a**, **4d**, **4e**, **4f** and **4h** have exhibited DPPH free radical scavenging activity at 25, 50, 75 and 100 mg/mL concentrations. Compound **4a** exhibited DPPH free radical scavenging activity 1.e. 7.27, 11.01, 23.11 and 32.12% at 25, 50, 75 and 100 mg/mL concentrations respectively. Compounds **4b** and **4c** have shown no DPPH free radical scavenging activity at all concentrations. Compounds **4d**, **4e** and **4f** exhibited DPPH free radical scavenging activity 1.e. 12.21, 32.67, 47.27, 52.67%, 16.36, 24.72 48.10, 61.47% and 7.51, 19.57, 31.45, 58.41% respectively. Compounds **4g** and **4i** have shown no DPPH free radical scavenging capacity at 25, 50, 75 and 100 mg/mL concentrations. Compound **4h** exhibited DPPH free radical scavenging activity 1.e. 6.21, 19.41, 21.42 and 34.36% at 25, 50, 75 and 100 mg/mL concentrations. From the antioxidant activity results, we conclude that compound **4e** displayed potent free radical scavenging activity 1.e. 61.47% at 100 µg/mL with least IC_{50} value 87.14 mg/mL. This result may be due to presence of nitro group on phenyl ring. The box plot and graphical representation of antioxidant activity of all synthesized compounds (**4a-4i**) were shown in Figs 10-11.

Conclusions

A simple and efficient synthesis of substituted pyrazoles (**4a-4i**) were reported in this paper *via* condensation reaction between substituted aldehydes (**1a-1i**), malononitrile (**2**) and 2,4-dinitrophenyl hydrazine (**3**) in presence of acylated Guar-gum. Solvent-free medium, non-toxic side products, inexpensive reagents and mild reaction conditions etc. are some beauties of present methodology. All synthesized compounds (**4a-4i**) were also tested for their bio efficacy in terms of herbicidal activity against *Raphanus sativus* L. (Radish) seeds, fungicidal activity against *R. solani*, *A. niger* and antioxidant activity was also evaluated. Based on activity results, it can be concluded that some of synthesized compounds possessed good activity due to presence of hydroxy, nitro groups on phenyl ring.

Declarations

Conflicts of interest

Authors declare that there is no conflict of interest regarding the publication of this paper.

Funding

Authors received no specific funding for this study. The funder has no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Acknowledgements

The authors are thankful to the Department of Chemistry, Chaudhary Charan Singh Haryana Agricultural University, Hisar for providing the necessary facilities. Financial assistance from Department of Science and Technology (DST), New Delhi, India is gratefully acknowledged. Authors are also thankful to SAIF, Punjab University Chandigarh, for providing analytical facilities for characterization of compounds.

Author Contributions

Conceptualization: Suman Sangwan, Rajvir Singh, Susheel Gulati, Suprita Rana

Data curation: Suman Sangwan, Rajvir Singh, Susheel Gulati

Formal analysis: Suman Sangwan, Rajvir Singh, Susheel Gulati

Investigation: Suman Sangwan, Rajvir Singh, Susheel Gulati, Suprita Rana

Methodology: Suman Sangwan, Susheel Gulati, Suprita Rana

Supervision: Suman Sangwan, Rajvir Singh, Susheel Gulati

Validation: Suman Sangwan, Rajvir Singh, Susheel Gulati

Writing-original draft: Suman Sangwan, Susheel Gulati,

Writing-review & editing: Suman Sangwan, Rajvir Singh, Susheel Gulati

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Figures

Figure 1

Substituted pyrazoles (4a-4i)

Figure 2

Box plot of substituted pyrazoles (4a-4i) against *Raphanus sativus* L. (root)

Figure 3

Herbicidal activity of substituted pyrazoles (4a-4i) against *Raphanus sativus* L. (root)

Figure 4

Box plot of substituted pyrazoles (4a-4i) against *Raphanus sativus* L. (shoot)

Figure 5

Herbicidal activity of substituted pyrazoles (4a-4i) against *Raphanus sativus* L. (shoot)

Figure 6

Box plot of substituted pyrazoles (4a-4i) against *Rhizoctonia solani*

Figure 7

Antifungal activity of substituted pyrazoles (4a-4i) against *Rhizoctonia solani*

Figure 8

Box plot of substituted pyrazoles (4a-4i) against *Aspergillus niger*

Figure 9

Antifungal activity of substituted pyrazoles (4a-4i) against *Aspergillus niger*

Figure 10

Box plot of substituted pyrazoles (4a-4i) for antioxidant activity

Figure 11

Antioxidant activity of substituted pyrazoles (4a-4i)

Figure 12

Scheme 1: Synthesis of substituted pyrazoles (4a-4i)